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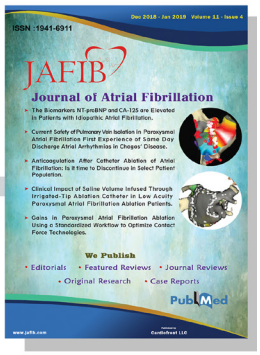


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# The Biomarkers NT-proBNP and CA-125 are Elevated in Patients with Idiopathic Atrial Fibrillation

Elton AMP Dudink*, Bob Weijs[1], Samantha Tull[2], Justin GLM Luermans[1], Larissa Fabritz[5], Winnie Chua[2], Michiel Rienstra[3], Isabelle C Van Gelder[3], Ulrich Schotten[4], Paulus Kirchhof[2,5,6], Harry JGM Crijns[1]

[1] *Maastricht University Medical Center+ and Cardiovascular Research Institute Maastricht (CARIM), Department of Cardiology, Maastricht, the Netherlands.*
[2] *University of Birmingham, Institute of Cardiovascular Sciences, Birmingham, United Kingdom.*
[3] *University Medical Center Groningen, Department of Cardiology, Groningen, the Netherlands.*
[4] *Maastricht University and Cardiovascular Research Institute Maastricht (CARIM), Department of Physiology, Maastricht, the Netherlands.*
[5] *Sandwell and West Birmingham Hospitals and University Hospitals Birmingham NHS trusts, Birmingham, United Kingdom.*
[6] *AFNET, Münster, Germany.*

## Abstract

**Background:** Blood biomarkers related to AF could be useful to detect silent AF and to develop stratified strategies for AF prevention. Previous studies identified markers that predict incident AF. However, it is difficult to differentiate whether biomarkers relate to underlying cardiovascular diseases, are generated by the atria in response to an AF episode, or both. We therefore measured a panel of blood biomarkers in patients without overt CVD with and without AF to investigate the association between biomarkers and atrial fibrillation (AF) in patients without overt cardiovascular disease (CVD).

**Methods:** Blood samples – drawn remote from an AF episode – of 60 patients with AF but without overt forms of CVD (idiopathic AF; iAF) were compared to 120 matched patients with sinus rhythm only. A novel antibody-based method for quantification of blood biomarkers (OlinkProseek Multiplex Cardiovascular) was used to compare 92 biomarkers between the two groups.

**Results:** N-terminal pro-B-type natriuretic peptide (NT-proBNP), Cathepsin L1, Endothelial cell-specific molecule 1, Cancer Antigen-125 (CA-125), Heat shock 27kDa protein, Galanin peptides, Proteinase-activated receptor 1, Stem cell factor, and CD40-ligand were all higher in iAF patients than in SR controls. Both NT-proBNP (OR1.55(1.07–2.25);p=0.022) and CA-125 (OR1.68(1.07–2.64);p=0.026) were independently associated with iAF.

**Conclusions:** This exploratory study, investigating over 90 cardiovascular blood biomarkers in patients without known CVD, identified one established biomarker for paroxysmal AF, NT-proBNP, and a novel marker, CA-125. CA-125 - previously unrelated to paroxysmal AF in an otherwise healthy population - may thus be a potential indicator of remote paroxysms of AF.

## Introduction

Atrial fibrillation (AF) usually occurs in patients with an increased vascular risk profile[1]. AF is called idiopathic AF (iAF) when meticulous phenotyping does not identify any concomitant (cardiovascular) disease. Therefore, these patients are most suitable to study early forms of this arrhythmia without the interference of associated disease[2].

In a recent report, Lind et al.[3] used the OlinkProseek Multiplex Cardiovascular I kit to identify blood markers associated with incident AF in two large-scale population studies. They concluded that N-terminal pro-B-type natriuretic peptide (NT-proBNP), Fibroblast growth factor 23 (FGF23), Fatty acid-binding protein 4 (FABP4), growth differentiation factor 15 (GDF-15), and Interleukin 6 (IL6) are determinants of incident AF, with NT-pro-BNP and FGF-23 remaining significant determinants upon correction for associated cardiovascular diseases. However, as these individuals were recruited from population studies, there were relatively high rates of hypertension and of history of stroke, myocardial infarction and heart failure.

In the study reported here, we used the OlinkProseek Multiplex Cardiovascular I kit to identify markers associated with the presence of AF in otherwise healthy patients, enabling identification of

## Key Words

Biomarkers,Atrial Fibrillation,Chronic Coronary Disease

**Corresponding Author**
Elton AMP Dudink,
Maastricht University Medical Centre+ and Cardiovascular Research Institute Maastricht (CARIM), Department of Cardiology 6202 AZ Maastricht, the Netherlands

markers associated with AF rather than with underlying vascular disease.

## Material and Methods

Of all consecutive patients who underwent cardiac computed tomography angiography (CTA) in the Maastricht University Medical Center between January 2008 and March 2011, all iAF patients were selected (n=115), and all age (±1 year), sex and PROCAM cardiovascular risk score-matched(±2%)[4] patients in permanent sinus rhythm (n=275) were selected as described previously[5]. All patients underwent CTA to detect subclinical coronary artery disease (sCAD). Out of these patients, EDTA-plasma was available for analysis in 180 patients (60 iAF, 120 SR). All patients were in sinus rhythm during blood sampling, which was performed after an overnight fast, at the day of CTA. The study was approved by the Institutional Review Board, all participants gave written informed consent. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Idiopathic AF was defined as the absence of hypertension (antihypertensive drug use, or systolic blood pressure ≥140 mm Hg, or diastolic blood pressure ≥90 mm Hg, or left ventricular hypertrophy [interventricular septum width >10 mm, posterior wall width >10 mm]), diabetes (fasting blood glucose >7.0 mmol/L), or hypercholesterolemia (total fasting cholesterol >7.0 mmol/L). In addition, none of the patients had a history of coronary artery disease, renal dysfunction, chronic heart failure, stroke, malignancy, thyroid disease or pulmonary disease, or evidence of structural cardiovascular disease on echocardiogram, including valvular heart disease (< grade 1).

In all blood samples, the OlinkProseek Multiplex Cardiovascular I kit (Olink Proteomics, Uppsala, Sweden) was used to measure proteins in EDTA plasma by real timePolymerase Chain Reaction[6]. Due to a low call rate (valid measurement in <85% of patients), Interleukin 4 (IL4), Natriuretic Peptides B (BNP), and Melusin (ITGB1BP2) were not included in further analyses. Values which were below the Limit of Detection (LOD) were replaced by the LOD value (http://www.olink.com/data-you-can-trust/validation/). Ten patients were not included in further analyses based on a large number of values below LOD (valid measurement for <85% of the proteins). The data from the panels were normalised to the median of 0 for each protein. This procedure enabled comparisons between the measurements from different panels. The panel provides NPX-values which allow for relative quantification, values can only be compared for the same protein across samples. NPX values for 2 different proteins are not comparable.

### Statistical analyses

Statistical analyses were performed using SPSS statistical software (IBM SPSS statistics version 23.0, IBM Corporation, Armonk, NY). Correlations are presented as Pearson's r and p-value. Normally distributed continuous variables are presented as mean±standard deviation, non-normally distributed continuous as median[interquartile range], and categorical variables as number of patients and percentage. All biomarkers were tested for an association with iAF using logistic regression, adjusting for age, sex (following the biomarker panel's instructions) and the presence of CT-angiographic sCAD. All significant biomarkers, age, sex and sCAD were included in multivariable logistic regression. Results were checked for collinearity and interaction among covariates, which were not found. Manual backwards elimination, retaining age, sex and sCAD was used to construct the final models (retention level set at p<0.10). Odds ratios and 95% confidence intervals were calculated. Overall, p<0.05 was considered significant. The first author had full access to all the data in the study and takes responsibility for its integrity and the data analysis.

## Results

As patients originate from a matched case control study, age (54.3±9.3 vs 54.1±10.3), sex (30.0% vs 31.7% women) and blood pressure (systolic blood pressure 126±9mmHg vs 126±12mmHg, diastolic blood pressure 80±10mmHg vs 78±10mmHg) did not differ significantly between patients with sinus rhythm and idiopathic AF. Echocardiographic parameters did not differ significantly (left atrial diameter 37±4mm vs 39±5mm, interventricular septum thickness 8.8±1.1mm vs 8.6±0.7mm, posterior wall thickness 8.7±1.1mm vs 8.5±0.6mm, and left ventricular ejection fraction 61[4]% vs 62[5]% respectively). The prevalence of sCAD was higher in the iAF group (36.5% vs 46.0%), while statin use was comparable (10.6% vs 11.7%). Full baseline characteristics and imaging parameters were reported earlier[5,7].

The age, sex and sCAD adjusted odds ratios of the 89 biomarkers and their 95% confidence interval (95%CI) are shown in Supplemental [Table 1]. N-terminal pro-B-type natriuretic peptide (NT-proBNP; OR1.70 (1.16–2.49), p=0.006), Cathepsin

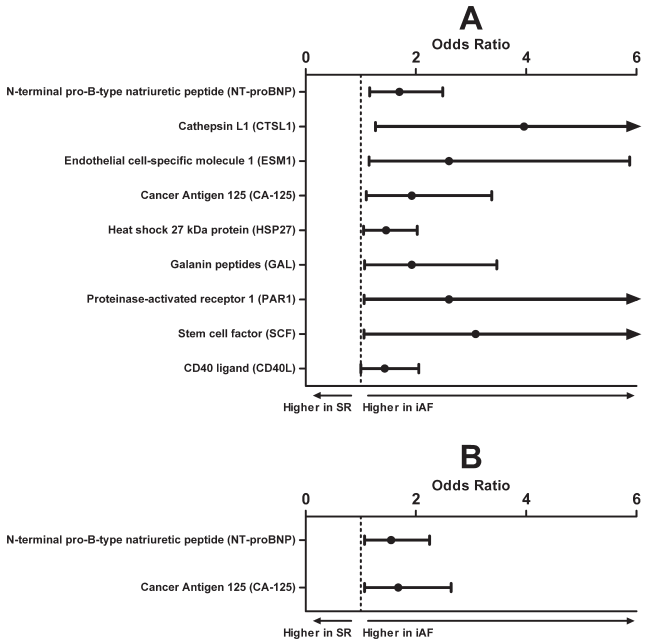


**Figure 1:** **Shown are age, sex and presence of sCAD-adjusted odds ratios and 95% confidence intervals. Panel A: Nine biomarkers differ significantly in idiopathic atrial fibrillation (iAF) compared to sinus rhythm controls (SR). Panel B: Multivariable regression analysis revealed that both NT-proBNP and CA-125 are independently associated with the presence of iAF.**

For all biomarkers shown p≤0.05.

**Table 1:** Odds ratios, corrected for age, sex and subclinical coronary artery disease (sCAD) on computed tomography, of 89 biomarkers of the idiopathic AF versus the sinus rhythm control group. Shown are OR(95% CI) and corresponding p-value

| | Age, sex and sCAD adjusted | |
|---|---|---|
| | OR (95% CI) | P |
| N-terminal pro-B-type natriuretic peptide (NT-proBNP) | 1.701 (1.163 – 2.488) | 0.006 |
| Cathepsin L1 (CTSL1) | 3.961 (1.269 – 12.356) | 0.018 |
| Endothelial cell-specific molecule 1 (ESM1) | 2.601 (1.151 – 5.876) | 0.022 |
| Cancer Antigen 125 (CA125) | 1.927 (1.101 – 3.375) | 0.022 |
| Heat shock 27 kDa protein (HSP27) | 1.459 (1.052 – 2.023) | 0.024 |
| Galanin peptides (GAL) | 1.925 (1.068 – 3.468) | 0.029 |
| Proteinase-activated receptor 1 (PAR1) | 2.601 (1.060 – 6.384) | 0.037 |
| Stem cell factor (SCF) | 3.082 (1.057 – 8.986) | 0.039 |
| CD40 ligand (CD40L) | 1.433 (1.000 – 2.055) | 0.050 |
| TNF-related apoptosis-inducing ligand (TRAIL) | 3.048 (0.952 – 9.759) | 0.061 |
| Platelet endothelial cell adhesion molecule (PECAM1) | 2.022 (0.943 – 4.334) | 0.070 |
| Pentraxin-related protein PTX3 (PTX3) | 1.774 (0.933 – 3.375) | 0.081 |
| Monocyte chemotactic protein 1 (MCP1) | 0.525 (0.248 – 1.113) | 0.093 |
| C-X-C motif chemokine 1 (CXCL1) | 1.387 (0.945 – 2.035) | 0.095 |
| Spondin 1 (SPON1) | 2.249 (0.840 – 6.026) | 0.107 |
| Tissue factor (TF) | 2.385 (0.809 – 7.029) | 0.115 |
| Beta-nerve growth factor (Beta-NGF) | 1.972 (0.839 – 4.637) | 0.120 |
| Pappalysin-1 (PAPPA) | 1.673 (0.853 – 3.284) | 0.134 |
| TNF-related activation-induced cytokine (TRANCE) | 1.531 (0.865 – 2.710) | 0.143 |
| E-selectin (SELE) | 0.649 (0.360 – 1.171) | 0.151 |
| Leptin (LEP) | 0.740 (0.483 – 1.134) | 0.167 |
| Interleukin-6 receptor subunit alpha (IL6RA) | 1.766 (0.762 – 4.091) | 0.185 |
| Fractalkine (CX3CL1) | 1.836 (0.740 – 4.559) | 0.190 |
| Matrix metalloproteinase 12 (MMP12) | 1.371 (0.842 – 2.230) | 0.204 |
| Proto-oncogene tyrosine-protein kinase Src (SRC) | 1.332 (0.847 – 2.097) | 0.215 |
| Follistatin (FS) | 1.502 (0.789 – 2.861) | 0.216 |
| Agouti-related protein (AGRP) | 1.493 (0.773 – 2.885) | 0.233 |
| Epidermal growth factor (EGF) | 1.207 (0.879 – 1.658) | 0.245 |
| Fibroblast growth factor 23 (FGF23) | 1.301 (0.833 – 2.030) | 0.247 |
| Myoglobin (MB) | 0.708 (0.390 – 1.285) | 0.256 |
| Adrenomedullin (AM) | 1.660 (0.685 – 4.021) | 0.261 |
| Interleukin 16 (IL16) | 1.457 (0.747 – 2.841) | 0.269 |
| Heparin-binding EGF-like growth factor (HB-EGF) | 1.637 (0.668 – 4.010) | 0.281 |
| Osteoprotegerin (OPG) | 1.572 (0.650 – 3.799) | 0.315 |
| Dickkopf-related protein 1 (DKK1) | 1.313 (0.764 – 2.257) | 0.325 |
| Myeloperoxidase (MPO) | 1.723 (0.578 – 5.133) | 0.329 |
| NF-kappa-B essential modulator (NEMO) | 1.234 (0.800 – 1.905) | 0.342 |
| Matrix metalloproteinase 3 (MMP3) | 1.308 (0.751 – 2.278) | 0.344 |
| ST2 protein (ST2) | 1.336 (0.729 – 2.450) | 0.348 |
| Interleukin-27 subunit alpha (IL27A) | 1.641 (0.573 – 4.697) | 0.356 |
| TNF-related apoptosis-inducing ligand receptor 2 (TRAILR2) | 0.615 (0.215 – 1.759) | 0.365 |
| P-selectin glycoprotein ligand 1 (PSGL-1) | 0.455 (0.082 – 2.528) | 0.368 |
| CD40L receptor (CD40) | 1.403 (0.670 – 2.939) | 0.369 |
| Placenta growth factor (PIGF) | 1.529 (0.566 – 4.130) | 0.402 |
| Membrane-bound aminopeptidase P (mAmP) | 0.877 (0.643 – 1.198) | 0.410 |
| Continue the table .. .. ... | | |
| Tumor necrosis factor receptor 2 (TNFR2) | 1.352 (0.619 – 2.954) | 0.450 |
| Thrombomodulin (TM) | 1.467 (0.520 – 4.135) | 0.469 |
| Interleukin 8 (IL8) | 0.794 (0.422 – 1.495) | 0.475 |
| Macrophage colony stimulating factor (CSF1) | 1.650 (0.408 – 6.673) | 0.482 |
| Tumor necrosis factor ligand superfamily member 14 (TNFSF14) | 1.353 (0.562 – 3.257) | 0.501 |
| Receptor for advanced glycosylation end products (RAGE) | 1.298 (0.549 – 3.071) | 0.552 |
| Growth/differentiation factor 15 (GDF-15) | 1.212 (0.607 – 2.419) | 0.586 |
| T-cell immunoglobulin and mucin domain 1 (TIM) | 0.873 (0.530 – 1.437) | 0.592 |
| Angiopoietin-1 receptor (TIE2) | 1.287 (0.421 – 3.935) | 0.658 |
| Interleukin 6 (IL6) | 0.922 (0.632 – 1.343) | 0.672 |
| Tissue-type plasminogen activator (tPA) | 1.150 (0.593 – 2.233) | 0.679 |
| Matrix metalloproteinase 1 (MMP1) | 0.902 (0.544 – 1.496) | 0.689 |
| Hepatocyte growth factor (HGF) | 1.154 (0.565 – 2.356) | 0.694 |
| Eosinophil cationic protein (ECP) | 1.112 (0.644 – 1.919) | 0.703 |
| C-C motif chemokine 4 (CCL4) | 1.106 (0.656 – 1.863) | 0.706 |
| Vascular endothelial growth factor D (VEGF-D) | 0.901 (0.505 – 1.606) | 0.723 |
| Prolactin (PRL) | 1.089 (0.678 – 1.747) | 0.725 |
| SIR2-like protiein (SIRT2) | 1.038 (0.821 – 1.038) | 0.757 |
| C-X-C motif chemokine 6 (CXCL6) | 0.935 (0.587 – 1.489) | 0.776 |
| Chitinase-3-like protein 1 (CHI3L1) | 0.939 (0.602 – 1.463) | 0.780 |
| Interleukin 1 receptor antagonist protein (IL1RA) | 0.924 (0.519 – 1.654) | 0.789 |
| Galectin 3 (GAL3) | 0.915 (0.463 – 1.810) | 0.799 |
| Tumor necrosis factor receptor superfamily member 6 (FAS) | 1.123 (0.445 – 2.835) | 0.806 |
| Urokinase plasminogen activator surface receptor (UPAR) | 1.119 (0.451 – 2.773) | 0.808 |
| Cathepsin D (CTSD) | 0.914 (0.436 – 1.915) | 0.811 |
| Kallikrein 11 (hK11) | 0.904 (0.390 – 2.093) | 0.814 |
| Kallikrein 6 (KLK6) | 1.097 (0.452 – 2.662) | 0.837 |
| Vascular endothelial growth factor A (VEGF-A) | 1.095 (0.400 – 3.000) | 0.859 |
| Cystatin B (CSTB) | 1.045 (0.641 – 1.703) | 0.860 |
| Fatty acid-binding protein 4 (FABP4) | 1.066 (0.521 – 2.183) | 0.861 |
| Lectin-like oxidized LDL receptor 1 (LOX1) | 1.043 (0.605 – 1.798) | 0.879 |
| C-C motif chemokine 20 (CCL20) | 0.978 (0.709 – 1.349) | 0.890 |
| Caspase 8 (CASP8) | 1.032 (0.639 – 1.668) | 0.897 |
| Resistin (RETN) | 0.962 (0.519 – 1.781) | 0.901 |
| Platelet-derived growth factor subunit B (PDGFsuB) | 1.015 (0.773 – 1.334) | 0.912 |
| Protein S100-A12 (EN-RAGE) | 0.969 (0.553 – 1.700) | 0.913 |
| Interleukin 18 (IL18) | 1.034 (0.530 – 2.018) | 0.921 |
| Growth hormone (GH) | 0.992 (0.844 – 1.166) | 0.924 |
| Matrix metalloproteinase (MMP10) | 1.027 (0.583 – 1.809) | 0.927 |
| C-C motif chemokine 3 (CCL3) | 1.044 (0.408 – 2.670) | 0.928 |
| Tumor necrosis factor receptor 1 (TNFR1) | 1.043 (0.373 – 2.918) | 0.937 |
| Renin (REN) | 1.004 (0.622 – 1.618) | 0.988 |
| C-X-C motif chemokine 16 (CXCL16) | 0.993 (0.338 – 2.923) | 0.990 |

L1 (CTSL1; OR3.96 (1.27–12.36, p=0.018), Endothelial cell-specific molecule 1 (ESM1; OR2.60 (1.15–5.88), p=0.022), Cancer Antigen 125 (CA-125; OR 1.93 (1.10–3.38), p=0.022), Heat shock 27 kDa protein (HSP27; OR1.46 (1.05–2.02), p=0.024), Galanin peptides (GAL; OR1.93 (1.07–3.47), p=0.029), Proteinase-activated receptor 1 (PAR1; OR2.60 (1.06–6.38), p=0.037), Stem cell factor (SCF; OR3.08 (1.06–8.99), p=0.039), and CD40 ligand (CD40L; OR1.43 (1.00–2.06), p=0.050) were all higher in iAF patients than in SR controls [Figure 1]. The mean expression of these 9 biomarkers is shown in [Figure 2]. There were no significant differences in the levels of the other biomarkers between iAF and controls.

Multivariable analysis showed that both CA-125 and NT-proBNP, adjusted for age, sex and sCAD, are associated with a history of iAF (OR for CA-125 2.25 (1.27-3.99), p=0.005 and NT-proBNP 1.79 (1.22-2.62), p=0.002). NT-proBNP and CA-125 do not correlate with each other (r=-0.09, p=0.27). [Figure 3] shows the prevalence of iAF in tertiles of the CA-125 (21.7%, 28.6% and 35.0%; p=0.27) and NT-proBNP levels (19.3%, 26.2% and 39.7%; p=0.04). The highest prevalence of iAF can be found in patients with levels of both CA-125 and NT-proBNP in the highest tertile (50.0%), with an intermediate prevalence in patients with either CA-125 (30.0%) or NT-proBNP (37.2%) in the highest tertile and the lowest prevalence in patients with none of the markers in the highest tertile (18.9%; p=0.005).

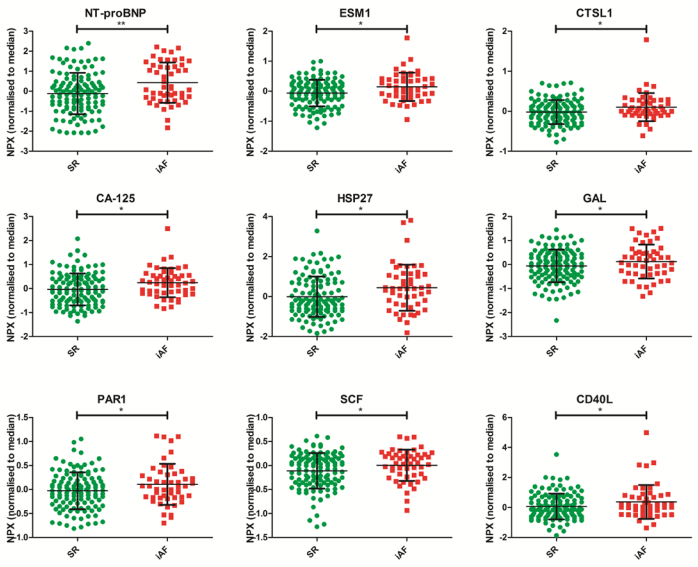


**Figure 2:** **Unadjusted box-plots of 9 biomarkers that were found to be significantly different between patients without overt cardiovascular disease, with and without atrial fibrillation. Shown are mean and standard deviations.**

** indicates p<0.01, * indicates p<0.05.
CA-125=Cancer Antigen 125; CD40L=CD40 ligand; CTSL1=Cathepsin L1 ; ESM1=Endothelial cell□specific molecule 1; GAL=Galanin peptides; HSP27=Heat shock 27 kDa protein; iAF=idiopathic atrial fibrillation, NPX=normalized protein expression, NT-proBNP=N□terminal pro□B□type natriuretic peptide; PAR1=Proteinase-activated receptor 1; SCF=Stem cell factor; SR=sinus rhythm.

## Discussion

From this study, it can be concluded that even in a healthy population without cardiovascular disease, the levels of NT-proBNP and CA-125 are associated with AF. Results from this study furthermore show that the levels of CTSL1, ESM1, HSP27, GAL, PAR1, SCF and CD40L may contain additional information on the presence of a history of AF.

Multivariable analysis showed that two markers are independently associated with the presence of a history of AF: NT-proBNP and CA-125. NT-proBNP has been recognized as a marker of incident AF in population studies, on top of risk scores based on the presence of cardiovascular disease[8,9]. The data from this cohort confirm that elevated NT-proBNP blood levels are a marker of (remote) episodes of paroxysmal AF even in the absence of concomitant vascular disease.

CA-125 is in current clinical use in the follow-up of ovarian cancer, but the value of CA-125 as a biomarker in cardiology is still to be determined[10]. CA-125 has before been linked – in small studies – to new-onset AF in patients with recompensated acute heart failure[11] as well as in post-menopausal women[12], but not yet to prevalent AF in a healthy population. There may be three possible explanations for the association between CA-125 and AF. Firstly, as CA-125 positively correlates with TNF-α, IL-6, IL-10[13] and IL-1β[14], it may be hypothesized to be a marker of the presence of underlying low-grade inflammation in this study population. Secondly: CA-125 is produced in response to mechanical stress or inflammation by different tissues derived from coelomic epithelium, such as the pericardium and pleura[10]. Based on the strong anatomical relation between the posterior left atrium and the pericardium[15] and the dominance of pulmonary vein triggers in early AF, the rise in CA-125 may be hypothesized to be a reflection of the interplay between atrium and pericardium. Lastly, as CA-125 is shown to correlate with pleural effusion[16], it may be hypothesized that the paroxysms of AF have led to – transient – pleural effusion with an associated rise in CA-125.Univariable regression analysis revealed that several pathophysiological processes may be active in patients with early forms of AF, such as angiogenesis (CTSL1[17], ESM1[18]), endothelial dysfunction (ESM1[18], SCF[19]), cellular stress (HSP27[20]), cardiac remodelling (CTSL1[20]), cardiac remodelling (CTSL1[21]) and activation of the coagulation system (GAL[22], PAR1[23], and CD40L[24]). The markers of a hypercoagulable state are in line withprevious reports of a prothrombotic state veryearly in the pathogenesis of AF[25,26]. Clearly, a much larger patient sample would be needed to verify any of these interactions which did not uphold during multivariable testing.

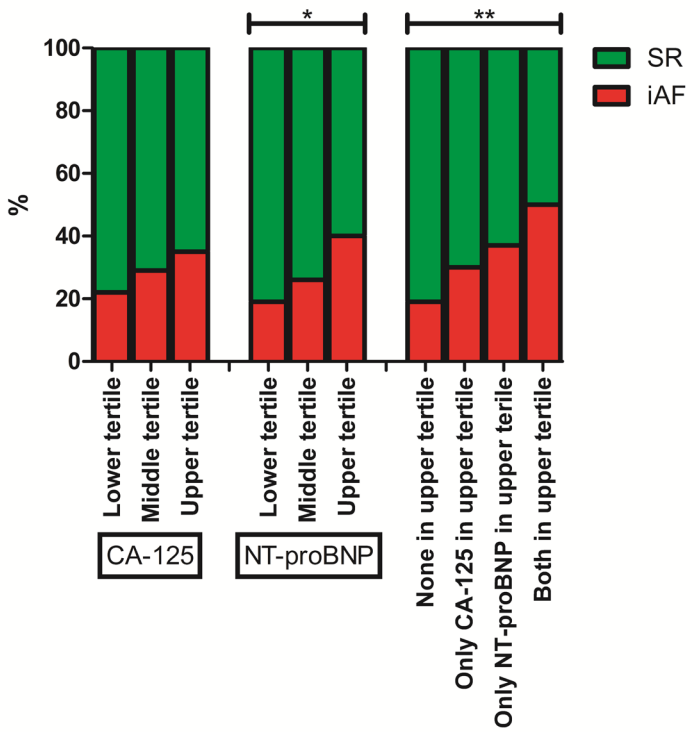


**Figure 3:** **Percentages of patients with idiopathic atrial fibrillation (iAF) in tertiles of CA-125 and NT-proBNP levels. SR=Sinus Rhythm**

* indicates P<0.05, ** indicates P<0.01.natriuretic peptide; PAR1=Proteinase-activated receptor 1; SCF=Stem cell factor; SR=sinus rhythm.

FGF-23 has been found to be associated with incident and prevalent AF in population studies[3,27-29]. We could not replicate this association despite shared methodology[3]. FGF-23 levels are clearly influenced by blood pressure, left ventricular mass, cardiovascular disease in general, and chronic kidney disease[30-33]. As patients with these conditions were excluded from the present analysis, we may conclude that FGF-23 is a marker of advanced vascular disease, while the markers found in this study are associated with very early AF without the effects of confounding vascular disease.

The current categorization of AF in paroxysmal, (long-standing) persistent or permanent may in the long run fall short to adequately describe distinct patient groups[34]. Biomarkers, be it those found in blood or through the use of imaging, could help determine and distinguish between different forms of AF. The results from the present study suggest that, when reporting on biomarkers in AF, underlying processes – such as the presence of other forms of cardiovascular disease - that lead to the creation of a substrate for AF should be taken into account.

### Strengths,limitations and clinical implications

The major strength of this study lies in the selection of patients without concomitant vascular disease and blood sampling during sinus rhythm, which enabled us to identify and gain further insight into markers associated with the presence of AF, rather than markers of the vascular conditions that usually accompany AF[35]. However, the study population of this exploratory study is relatively small and highly selected, necessitating confirmation in larger study populations. Next to more insight in the pathophysiology of early AF without concomitant vascular disease, this study should be seen as a means to support investigating the use of NT-proBNP and CA-125 in screening programs for AF incorporating biomarkers[36], which should be tested prospectively.

## Sources of Funding

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## Conclusion

This study confirms the association between levels of NT-proBNP and a diagnosis of paroxysmal AF even in patients without concomitant cardiovascular conditions, suggesting that NT-proBNP is released by the atria and remains elevated even in the absence of an on-going arrhythmia episode. Furthermore, this data suggests that CA-125 could be a novel marker of the occurrence of (paroxysms of) AF in patients without structural heart disease. Finally, in conjunction with published analyses, our data suggest that elevated FGF-23 levels may be a marker for AF in patients with concomitant cardiovascular disease.

## References

1. Nieuwlaat R, Prins MH, Le Heuzey JY, Vardas PE, Aliot E, Santini M, Cobbe SM, Widdershoven JW, Baur LH, Levy S, Crijns HJ. Prognosis, Disease Progression, and Treatment of Atrial Fibrillation Patients During 1 Year: Follow-up of the Euro Heart Survey on Atrial Fibrillation. Eur Heart J. 2008;29(9):1181-9.
2. Linz D, Elliott AD, Marwick TH, Sanders P. Biomarkers and New-Onset Atrial Fibrillation to Assess Atrial Cardiomyopathy. Int J Cardiol. 2017;248:208-10.
3. Lind L, Sundstrom J, Stenemo M, Hagstrom E, Arnlov J. Discovery of New Biomarkers for Atrial Fibrillation Using a Custom-Made Proteomics Chip. Heart. 2017;103(5):377-82.
4. Assmann G, Cullen P, Schulte H. Simple Scoring Scheme for Calculating the Risk of Acute Coronary Events Based on the 10-Year Follow-up of the Prospective Cardiovascular Munster (Procam) Study. Circulation. 2002;105(3):310-5.
5. Weijs B, Pisters R, Haest RJ, Kragten JA, Joosen IA, Versteylen M, Timmermans CC, Pison L, Blaauw Y, Hofstra L, Nieuwlaat R, Wildberger J, Crijns HJ. Patients Originally Diagnosed with Idiopathic Atrial Fibrillation More Often Suffer from Insidious Coronary Artery Disease Compared to Healthy Sinus Rhythm Controls. Heart Rhythm. 2012;9(12):1923-9.
6. Assarsson E, Lundberg M, Holmquist G, Bjorkesten J, Thorsen SB, Ekman D, Eriksson A, Rennel Dickens E, Ohlsson S, Edfeldt G, Andersson AC, Lindstedt P, Stenvang J, Gullberg M, Fredriksson S. Homogenous 96-Plex Pea Immunoassay Exhibiting High Sensitivity, Specificity, and Excellent Scalability. PLoS One. 2014;9(4):e95192.
7. Dudink E, Peeters F, Altintas S, Heckman LIB, Haest RJ, Kragten H, Kietselaer B, Wildberger J, Luermans J, Weijs B, Crijns H. Agatston Score of the Descending Aorta Is Independently Associated with Coronary Events in a Low-Risk Population. Open Heart. 2018;5(2):e000893.
8. Patton KK, Heckbert SR, Alonso A, Bahrami H, Lima JA, Burke G, Kronmal RA. N-Terminal Pro-B-Type Natriuretic Peptide as a Predictor of Incident Atrial Fibrillation in the Multi-Ethnic Study of Atherosclerosis: The Effects of Age, Sex and Ethnicity. Heart. 2013;99(24):1832-6.
9. Sinner MF, Stepas KA, Moser CB, Krijthe BP, Aspelund T, Sotoodehnia N, Fontes JD, Janssens AC, Kronmal RA, Magnani JW, Witteman JC, Chamberlain AM, Lubitz SA, Schnabel RB, Vasan RS, Wang TJ, Agarwal SK, McManus DD, Franco OH, Yin X, Larson MG, Burke GL, Launer LJ, Hofman A, Levy D, Gottdiener JS, Kaab S, Couper D, Harris TB, Astor BC, Ballantyne CM, Hoogeveen RC, Arai AE, Soliman EZ, Ellinor PT, Stricker BH, Gudnason V, Heckbert SR, Pencina MJ, Benjamin EJ, Alonso A. B-Type Natriuretic Peptide and C-Reactive Protein in the Prediction of Atrial Fibrillation Risk: The Charge-Af Consortium of Community-Based Cohort Studies. Europace. 2014;16(10):1426-33.
10. Bischof P. What Do We Know About the Origin of Ca 125? Eur J Obstet Gynecol Reprod Biol. 1993;49(1-2):93-8.
11. Yucel H, Kaya H, Zorlu A, Yildirimli K, Sancakdar E, Gunes H, Kurt R, Ozgul U, Turgut OO, Yilmaz MB. Cancer Antigen 125 Levels and Increased Risk of New-Onset Atrial Fibrillation. Herz. 2015;40 Suppl 2:119-24.
12. Sekiguchi H, Shimamoto K, Takano M, Kimura M, Takahashi Y, Tatsumi F, Watanabe E, Jujo K, Ishizuka N, Kawana M, Hagiwara N. Cancer Antigen-125 Plasma Level as a Biomarker of New-Onset Atrial Fibrillation in Postmenopausal Women. Heart. 2017.
13. Kosar F, Aksoy Y, Ozguntekin G, Ozerol I, Varol E. Relationship between Cytokines and Tumour Markers in Patients with Chronic Heart Failure. Eur J Heart Fail. 2006;8(3):270-4.
14. Stanciu AE, Stanciu MM, Vatasescu RG. Nt-Probnp and Ca 125 Levels Are Associated with Increased Pro-Inflammatory Cytokines in Coronary Sinus Serum of Patients with Chronic Heart Failure. Cytokine. 2018;111:13-9.

15. Yang F, Tiano J, Mittal S, Turakhia M, Jacobowitz I, Greenberg Y. Towards a Mechanistic Understanding and Treatment of a Progressive Disease: Atrial Fibrillation. J Atr Fibrillation. 2017;10(3):1627.
16. Li S, Ma H, Gan L, Ma X, Wu S, Li M, Tang CH, Tsai HC. Cancer Antigen-125 Levels Correlate with Pleural Effusions and Copd-Related Complications in People Living at High Altitude. Medicine (Baltimore). 2018;97(46):e12993.
17. Urbich C, Heeschen C, Aicher A, Sasaki K, Bruhl T, Farhadi MR, Vajkoczy P, Hofmann WK, Peters C, Pennacchio LA, Abolmaali ND, Chavakis E, Reinheckel T, Zeiher AM, Dimmeler S. Cathepsin L Is Required for Endothelial Progenitor Cell-Induced Neovascularization. Nat Med. 2005;11(2):206-13.
18. Rocha SF, Schiller M, Jing D, Li H, Butz S, Vestweber D, Biljes D, Drexler HC, Nieminen-Kelha M, Vajkoczy P, Adams S, Benedito R, Adams RH. Esm1 Modulates Endothelial Tip Cell Behavior and Vascular Permeability by Enhancing Vegf Bioavailability. Circ Res. 2014;115(6):581-90.
19. Wigren M, Rattik S, Hultman K, Bjorkbacka H, Nordin-Fredrikson G, Bengtsson E, Hedblad B, Siegbahn A, Goncalves I, Nilsson J. Decreased Levels of Stem Cell Factor in Subjects with Incident Coronary Events. J Intern Med. 2016;279(2):180-91.
20. Huot J, Houle F, Spitz DR, Landry J. Hsp27 Phosphorylation-Mediated Resistance against Actin Fragmentation and Cell Death Induced by Oxidative Stress. Cancer Res. 1996;56(2):273-9.
21. Sun M, Chen M, Liu Y, Fukuoka M, Zhou K, Li G, Dawood F, Gramolini A, Liu PP. Cathepsin-L Contributes to Cardiac Repair and Remodelling Post-Infarction. Cardiovasc Res. 2011;89(2):374-83.
22. Tyrrell C, Toyooka A, Khan F, Thornburg KL, Mudd JO, Hasan W. The Neuropeptide Galanin Promotes an Anti-Thrombotic Phenotype on Endocardial Endothelial Cells from Heart Failure Patients. Auton Neurosci. 2017;206:35-42.
23. Alberelli MA, De Candia E. Functional Role of Protease Activated Receptors in Vascular Biology. Vascul Pharmacol. 2014;62(2):72-81.
24. Pamukcu B, Lip GY, Snezhitskiy V, Shantsila E. The Cd40-Cd40l System in Cardiovascular Disease. Ann Med. 2011;43(5):331-40.
25. Hobbelt AH, Spronk HM, Crijns HJ, Ten Cate H, Rienstra M, Van Gelder IC. Prethrombotic State in Young Very Low-Risk Patients with Atrial Fibrillation. J Am Coll Cardiol. 2017;69(15):1990-2.
26. Spronk HM, De Jong AM, Verheule S, De Boer HC, Maass AH, Lau DH, Rienstra M, van Hunnik A, Kuiper M, Lumeij S, Zeemering S, Linz D, Kamphuisen PW, Ten Cate H, Crijns HJ, Van Gelder IC, van Zonneveld AJ, Schotten U. Hypercoagulability Causes Atrial Fibrosis and Promotes Atrial Fibrillation. Eur Heart J. 2017;38(1):38-50.
27. Mathew JS, Sachs MC, Katz R, Patton KK, Heckbert SR, Hoofnagle AN, Alonso A, Chonchol M, Deo R, Ix JH, Siscovick DS, Kestenbaum B, de Boer IH. Fibroblast Growth Factor-23 and Incident Atrial Fibrillation: The Multi-Ethnic Study of Atherosclerosis (Mesa) and the Cardiovascular Health Study (Chs). Circulation. 2014;130(4):298-307.
28. Alonso A, Misialek JR, Eckfeldt JH, Selvin E, Coresh J, Chen LY, Soliman EZ, Agarwal SK, Lutsey PL. Circulating Fibroblast Growth Factor-23 and the Incidence of Atrial Fibrillation: The Atherosclerosis Risk in Communities Study. J Am Heart Assoc. 2014;3(5):e001082.
29. Chua W, Purmah Y, Cardoso VR, Gkoutos GV, Tull SP, Neculau G, Thomas MR, Kotecha D, Lip GYH, Kirchhof P, Fabritz L. Data-Driven Discovery and Validation of Circulating Blood-Based Biomarkers Associated with Prevalent Atrial Fibrillation. Eur Heart J. 2018(In press.).
30. Verzola D, Ansaldo F, Milanesi S, Parodi EL, Rosa GM, Sofia A, Bonanni A, Viazzi F, Balbi M, Garibotto G. Interorgan Handling of Fibroblast Growth Factor-23 in Humans. Am J Physiol Renal Physiol. 2017;312(2):F254-F8.
31. Kestenbaum B, Sachs MC, Hoofnagle AN, Siscovick DS, Ix JH, Robinson-Cohen C, Lima JA, Polak JF, Blondon M, Ruzinski J, Rock D, de Boer IH. Fibroblast Growth Factor-23 and Cardiovascular Disease in the General Population: The Multi-Ethnic Study of Atherosclerosis. Circ Heart Fail. 2014;7(3):409-17.
32. Scialla JJ, Wolf M. Roles of Phosphate and Fibroblast Growth Factor 23 in Cardiovascular Disease. Nat Rev Nephrol. 2014;10(5):268-78.
33. Hennersdorf MG, Schueller PO, Steiner S, Strauer BE. Prevalence of Paroxysmal Atrial Fibrillation Depending on the Regression of Left Ventricular Hypertrophy in Arterial Hypertension. Hypertens Res. 2007;30(6):535-40.
34. Fabritz L, Guasch E, Antoniades C, Bardinet I, Benninger G, Betts TR, Brand E, Breithardt G, Bucklar-Suchankova G, Camm AJ, Cartlidge D, Casadei B, Chua WW, Crijns HJ, Deeks J, Hatem S, Hidden-Lucet F, Kaab S, Maniadakis N, Martin S, Mont L, Reinecke H, Sinner MF, Schotten U, Southwood T, Stoll M, Vardas P, Wakili R, West A, Ziegler A, Kirchhof P. Expert Consensus Document: Defining the Major Health Modifiers Causing Atrial Fibrillation: A Roadmap to Underpin Personalized Prevention and Treatment. Nat Rev Cardiol. 2016;13(4):230-7.
35. Ko D, Riles EM, Marcos EG, Magnani JW, Lubitz SA, Lin H, Long MT, Schnabel RB, McManus DD, Ellinor PT, Ramachandran VS, Wang TJ, Gerszten RE, Benjamin EJ, Yin X, Rienstra M. Metabolomic Profiling in Relation to New-Onset Atrial Fibrillation (from the Framingham Heart Study). Am J Cardiol. 2016;118(10):1493-6.
36. Svennberg E, Henriksson P, Engdahl J, Hijazi Z, Al-Khalili F, Friberg L, Frykman V. N-Terminal Pro B-Type Natriuretic Peptide in Systematic Screening for Atrial Fibrillation. Heart. 2017.